

EFFECT OF CHLORTETRACYCLINE ON THE DISTRIBUTION OF RESISTANCE GENES

Kim Baskerville and Julie A. Funk*

Department of Veterinary Preventive Medicine, College of Veterinary Medicine, The Ohio State University, Columbus, OH 43210. Phone: 614-247-6635, Fax: 614-292-4142, Email: funk.74@osu.edu

Abstract The objective of this study was to compare the distribution of tetracycline resistance genes in the Gram-negative fecal flora between swine that were fed subtherapeutic chlortetracycline as compared to pigs that did not receive in feed antimicrobials. Within each of 2 farms, 8 finisher barns were temporarily matched in pairs based on pig placement dates. For each matched pair, a barn was assigned to either treatment (50g chlortetracycline/ton of feed) or control. From 48 pigs per barn, 2 Gram negative colonies from the fecal flora were selected per pig for tetracycline resistance gene detection. The isolates were assayed for tet (A), (B), (C), (D), (E), and (G). A total of 88.7% of isolates (805/908) harboured at least one of the genes assayed. Overall, 35.3%, 59.9%, 0.9% of isolates were identified to harbour tet (A), (B), and (C), respectively. There were no differences detected in tetracycline resistance genes distribution between treatments.

Introduction Previous investigators have suggested that antimicrobial use policies on farms result in differential selection of tetracycline resistance genes in the bacterial flora (Bryan *et al.*, 2004; Lanz *et al.*, 2003; Lee *et al.*, 1993; Sunde *et al.*, 1998). These studies were limited by subjective measurements of general antimicrobial use (high or low use) or comparisons between different animal species. In none of the studies was tetracycline exposure evaluated. The objective of this research was to conduct a controlled clinical trial to evaluate the effect of sub-therapeutic chlortetracycline on the distribution of tetracycline resistance genes in the Gram-negative fecal flora of swine.

Materials and Methods Within each of 2 farms, treatments were assigned to temporally matched finisher barn pairs based on pig placement dates. For each matched pair, a barn was assigned to treatment (50g chlortetracycline/ton of feed) or control (no antibiotics in the feed). A total of 8 barns were enrolled. Pigs were fed the diets from 10 weeks of age until 2 weeks pre-market. Prior to withdrawal of chlortetracycline, fecal samples were collected from 48 individual pigs per barn prior to slaughter. Matched barn pairs were sampled on the same day. Fecal samples were plated onto MacConkey agar and at least 2 isolates were selected per pig. Multiplex PCR was used to detect six classes of tetracycline resistance determinants [tet (A), tet (B), tet (C), tet (D) tet (E), and tet (G)] as described by Ng (*et al.*, 2001).

The data was tabulated in a 2 (chlortetracycline or no chlortetracycline) X 4 (tet (A), tet (B), tet (C), tet (A) and (B) and no gene detected) table and the χ^2 test was calculated in order to compare the difference in distribution between the 2 treatment groups.

Results Overall, 908 isolates were assayed. A total of 88.7% of isolates (805/908) harboured at least one of the tetracycline resistance genes assayed. Overall, 35.3%, 59.9%, 0.9% of isolates were identified to harbour tet (A), (B), and (C), respectively. Both tet (A) and tet (B) were identified in 7.5% of isolates. For those swine that received chlortetracycline, 37.5%, 60.6%, 1.7% were identified to harbour tet (A), (B), and (C), respectively. For those swine that received no antibiotics 33.1%, 59.2%, 0.2% were identified to harbour tet (A), (B), and (C) respectively. There was no significant effect of treatment on the distribution of tetracycline resistance determinants ($p>0.10$).

Discussion These results suggest that subtherapeutic chlortetracycline, at least under short-term exposure, does not alter the distribution of tetracycline resistance genes in the fecal flora of swine. The predominant identification of tet (A), (B), and (C) in the Gram-negative fecal flora of swine is in agreement with previous reports. Although our results cannot discredit the potential

effect of selection pressure resultant to tetracycline use policies over several years, it does suggest that factors other than tetracycline exposure may be important for the differential distribution of tetracycline resistance genes between different animal species (Bryan *et al.*, 2004; Lanz *et al.*, 2003) or between swine herds with different antimicrobial use policies (Lee *et al.*, 1993; Sunde *et al.*, 1998). Future investigations of antimicrobial resistance genetics in animal populations should link specific antimicrobial use data, as well as evaluation of potential non-antimicrobial selection pressures, that may impact resistance gene emergence and dissemination.

References

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